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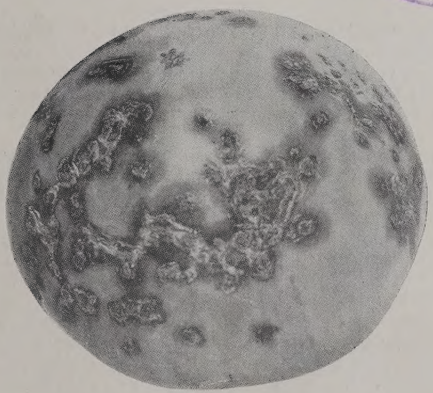

Fig. 1. Bacterial spot of tomato.

TOMATO BACTERIAL SPOT AND SEED DISINFECTION

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SUMMARY

Bacterial spot is a serious disease of tomatoes in Indiana and other states.

On the tomato fruit it has been called "scab," "small pox" and "canker."

Practically all varieties of tomatoes are susceptible. Peppers are also susceptible.

The chief economic importance of this disease is due to its attack upon the fruit. The blackened scab-like spots constitute a severe blemish from the market standpoint and are particularly objectionable to canners.

Spotted fruits are unsuitable for canning stock but can be used for catsup pulp.

Except upon seedlings and very young leaves, foliage infection does not cause noticeable damage.

The bacteria which cause this disease are highly resistant to drying and have been found to survive a year dried on tomato seed. These organisms are killed by a five-minute immersion in 1 to 3000 corrosive sublimate.

Foliage infection is readily obtained by spraying the plants with a water suspension of the bacteria. The spots become visible 3 to 5 days after inoculation. The bacteria enter the leaf through the stomata or breathing pores.

There are no stomata in the fruit epidermis and fruit infection is obtained only through wounds. Very minute puncture wounds permit infection and in the field insects probably cause the wounds through which infection occurs.

Green fruit is readily infected but it has been impossible to infect ripe fruit, probably because of its higher acidity.

The bacteria pass the winter dried on the surface of tomato seed and are thus disseminated. Even two-year-old seed may carry infection.

Contamination of the seed occurs during the process of seed extraction.

Seedling infection results from the use of contaminated seed. The disease spreads in the plant bed and is further disseminated with infected transplants.

In the field the disease is spread by the splashing and washing of rain. From the foliage the disease is spread to the fruit.

As a control measure it is recommended that tomato seed be treated in a 1 to 3000 corrosive sublimate solution 5 minutes and washed 10 to 15 minutes in running water.

TOMATO BACTERIAL SPOT AND SEED DISINFECTION

MAX W. GARDNER AND JAMES B. KENDRICK

The tomato crop of Indiana and neighboring states has been rather commonly affected with a spot disease of the fruit and foliage quite unlike any of the well-known tomato troubles. The attack on the fruit has been so conspicuous that growers and canners are already more or less familiar with the disease under such names as "scab" and "small pox." The prevalence and importance of this disease in the Indiana canning crop was first recognized by W. A. Huelsen of the Department of Horticulture as a result of his observations in 1918. Not until the present studies were begun was there any extensive investigation of this disease in this country. Observations were made on the field crop during the fall of 1919, experimental work was carried out during the following winter in the greenhouse and laboratory at Lafayette and field study was continued during the 1920 season. As a result of this work the cause and nature of the disease which has been named bacterial spot have been determined and a method of control devised.[1]

PREVIOUS INVESTIGATIONS

There is very little in the literature of plant pathology relative to this disease. It was recognized as a new trouble by Nelson who found it in 1917 in Tennessee, Illinois and Michigan and later Coons and Nelson[2, 3] in Michigan conducted successful inoculation tests and briefly described the trouble as tomato canker. Sherbakoff[4] at the Florida station briefly described a disease of peppers in 1917 which we now believe to be closely related to this tomato disease. Miss Doidge[5] has recently described this disease as it occurred in the market gardens of Pretoria in South Africa and has studied the bacteria which cause the disease.

OCCURRENCE OF THE DISEASE

Bacterial spot was very serious in the Indiana canning crop in 1918 and in 1919 the disease was found prevalent about Frankfort and Indianapolis during the latter part of September. It was not found in the Paoli region, however. During the 1920 season the disease was prevalent in the canning crop of central and southern Indiana and was noted about Lafayette, Indianapolis, Tipton, Kokomo, Shelbyville, Greenwood, Campbellsburg and in shipments from Dupont, Mitchell, and Medora. The disease

(1) GARDNER, MAX W. and KENDRICK, JAMES B. Bacterial spot of tomato. Jour. Agr. Research *21*: 123-156. 1921.

(2) COONS, G. H. Michigan plant disease survey for 1917. Mich. Acad. Sci. Rep. *20*: 425-450. 1918.

(3) COONS, G. H. and NELSON, RAY. Plant diseases of importance in the transportation of fruits and vegetables. Amer. Ry. Perishable Freight Ass'n Circ. 473-A: 1-64. 1918.

(4) SHERBAKOFF, C. D. Report of associate plant pathologist. Fla. Agr. Expt. Sta. Rep. *1917*: 76R-86R.

(5) DOIDGE, ETHEL M. A tomato canker. Jour. Dept. Agr. Union of South Africa *1*: 718-721. 1920. *Also* Annals of Applied Biology 7: 407-430. 1921.

was noted in several market gardens about Indianapolis and was also reported on Florida tomatoes in the Chicago market by G. K. K. Link. F. E. Parsons of the Federal Bureau of Markets reports that bacterial spot of tomatoes was severe at Flint, Texas, in 1917, and that it occurred near Dania, Florida, in 1918, 1919 and 1920.

In May, 1920, the disease was found in fields of tomato seedlings near Tifton, Ga., which were being grown for shipment to Indiana canners. As early as July 13, bacterial spot was found in rather advanced stages in one field near Kokomo, Indiana, which was planted with transplants grown in Georgia. Although the disease does appear and spread in plant beds in the north, the above observations tend to indicate that it is liable to gain much more headway under southern conditions and hence become more destructive in the northern fields in which southern-grown plants are used.

HOW LOSS IS CAUSED

Except among seedlings foliage infection does not do serious damage and the chief economic importance of bacterial spot is due to its attack upon the fruit. The disease lesions on the fruit constitute a very serious blemish from the market point of view since scabbed fruits are more or less unsalable. From the canners' point of view this disease is very objectionable. Spotted fruits are not suitable for first grade canning stock and can be used only in catsup pulp. The older lesions are not readily removed with the skin. Observations made upon the tomatoes passing over the sorting belts in canning factories have shown that during the latter part of the season 3 to 5 per cent of the tomatoes may be affected with bacterial spot. This disease in itself does not cause a rot of the fruit but the lesions do afford entrance to rot-producing organisms. In the growing crop the disease varies in prevalence and fields have been noted in the Indianapolis region in which 25 to 50 per cent of the fruits were spotted. Inasmuch as the quality of the tomatoes is seriously damaged, bacterial spot is a loss-producing factor of very considerable importance especially to the canners.

VARIETIES AFFECTED

In the commercial crop the disease has been noted on the varieties Greater Baltimore, Stone, Century, Arlington, Norton, Marvel, Columbia and Yellow Plum. In the greenhouse, foliage inoculation has been successful on 34 varieties as follows: Bloomsdale, Magnus, Paragon, Landreth, Delaware Beauty, John Baer, Hummer, Improved Trophy, Coreless, Success, Red Rock, Bonny Best, Mississippi Girl, Globe, Chalk's Early Jewel, Matchless, Favorite, Comet, Golden Queen, Acme, Perfection, Buckeye State, Earliana, June Pink, Ponderosa, Early Detroit, Improved Dwarf Champion, Yellow Pear-shaped, Red Pear-shaped, Dwarf Yellow Prince, Yellow Plum, Golden Ball, Yellow Peach and Red Cherry. In a field plot containing these 34 varieties, fruit infection occurred on all except the Golden Ball and the Red Pear-shaped. The variety Magnus seemed to be particularly susceptible.

SYMPTOMS ON THE FRUIT

Bacterial spot lesions on the fruit first appear as black raised points or dots with a narrow water-soaked border (Fig. 2 D). These enlarge to

form slightly elevated, blackened scabs with irregularly lobed margins about each of which there is usually a distinct water-soaked halo in the green tissue (Fig. 4 B). Usually the central tissue becomes brown, corky and fibrous, and distinctly scab-like in appearance (Fig. 1, and Fig 2, B and C). In Fig. 4 A, lesions of this type are greatly enlarged to show the corky central tissue, the ruptured epidermis of the fruit and the water-soaked halo. The central tissue may collapse so that the spots become sharply sunken pits or craters as shown in figures 2 A and 2 E. On ripe fruit the water-soaked border is absent or is replaced by a zone in which the green color persists. The spots about the stem end of the tomato are usually larger than those located elsewhere. Rather large scabby areas may result from the coalescence of several spots (Fig. 1) but, as a rule, no marked malformation or cracking of the fruit results. The lesions are

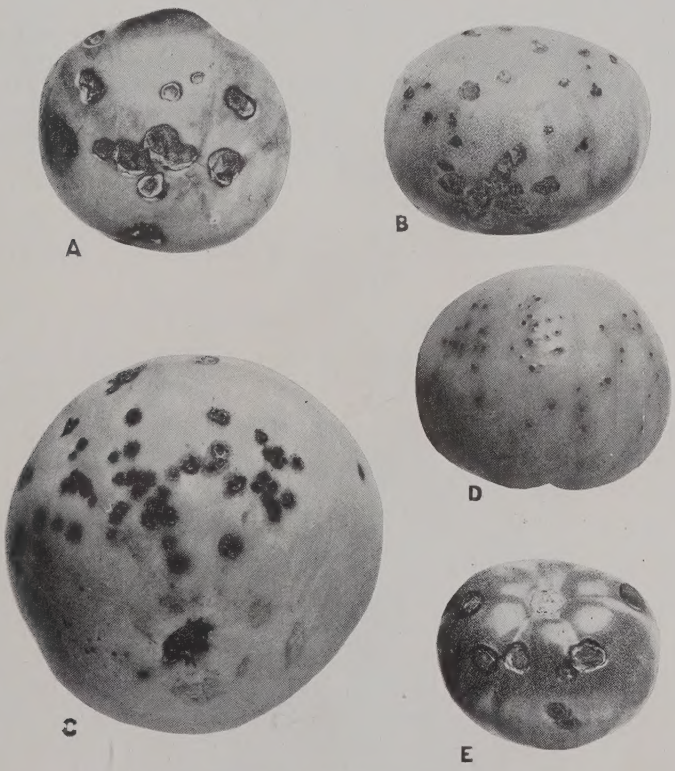

Fig. 2. Types of fruit infection.
A. Old, sunken lesions.
B. Young lesions on green fruit.
C. Scab-like spots on a ripe fruit.
D. Very young, raised spots.
E. Large, sunken lesions on a green fruit.

more or less superficial and do not penetrate through the fruit wall into the pulp.

Often there is found associated with this disease another type of fruit spot, a small circular white spot with a darker raised center, which has been called "white spot" or "bird's-eye spot" and is not to be confused with bacterial spot.

SYMPTOMS ON THE LEAVES

On the leaves bacterial spot infection produces at first very small water-soaked dots which are translucent when viewed in transmitted light. These enlarge and the centers become black and distinctly sunken on the lower side (Fig. 5) and later parchment-like with a tendency to crack (Fig. 6 A). These spots are not delimited by the veins as is the case in many bacterial leaf diseases, but are irregularly circular with a narrow yellowish translucent border. Thickset infection causes whole areas of the leaf to turn yellow or become dry and brown. Infection of young leaves causes distortion or even death of the leaflets and the lesions on young leaves are usually larger than those on old leaves (Figs. 6 A and 6 B).

It is very difficult to distinguish between these spots and those of the Septoria leaf-spot disease. The bacterial spots differ in that they are black and shiny on the upper surface, often crack in the center and lack the black points or pycnidia characteristic of Septoria.

Elongated black lesions are formed on stems and petioles. Cotyledon lesions are small sunken pits, at first silvery, later black in color (Fig. 6 C) and are at first visible on only one surface of the cotyledon. Badly affected cotyledons turn yellow prematurely.

CAUSE OF THE DISEASE

Small pieces of the diseased tissue from leaf and fruit lesions were crushed in drops of sterile water and from these drops agar plates were

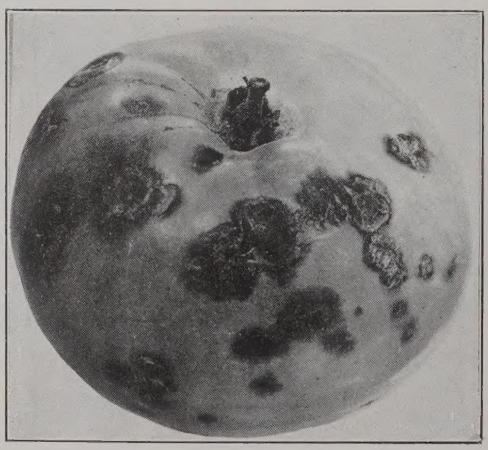

Fig. 3. Diseased fruit showing larger lesions near stem end.

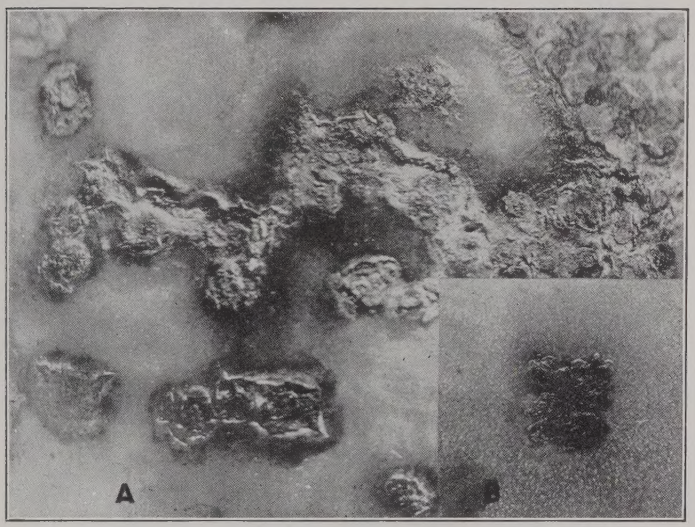

Fig. 4. Fruit lesions highly magnified.
A. Old lesions coalescing to form scabby areas on the fruit.
B. Young lesion with raised black scab-like center.

poured. In all cases numerous, glistening, yellow, translucent, bacterial colonies of the same type were obtained (Fig. 7). Inoculation of tomato plants in the greenhouse with bacteria from these colonies resulted in characteristic bacterial spot infection and the same type of bacteria was reisolated.

Further study of this parasite in pure culture in which it was grown on a variety of culture media and under a variety of conditions showed that it was an undescribed species of bacteria.[1] It is a rod-shaped organism with one polar flagellum (Fig. 8) closely resembling the bacteria that cause cabbage black rot and citrus canker. In culture it does not produce acid or gas and is very sensitive to acidity. It will develop in a wide range of temperatures but apparently is favored by temperatures in the neighborhood of 25° C. (77° F.) It is readily killed by a ten-minute immersion in water at a temperature of 50° C. (122° F.), a five-minute immersion in a 1 to 4000 corrosive sublimate solution and by a twenty-minute exposure to sunlight. The organism survives several days freezing in water, but is killed by long exposures. This organism is very resistant to desiccation and has survived 25 days dried on glass and twelve months on the surface of tomato seed.

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[1] The writers in their article submitted August, 1920, for publication in the Journal of Agricultural Research (l. c.) applied the name *Bacterium exitiosum* n. sp. to this organism. Before this article was printed, however, the work of Miss Doidge (l. c.) in South Africa appeared in which she described what is in all probability the same organism under the name *Bacterium vesicatorium* n. sp. If these organisms are identical, precedence should be given to the latter name.

Fig. 5. Lower epidermis of tomato leaflet showing sunken lesions of bacterial spot. Highly magnified.

INOCULATION TESTS

Infection of tomato foliage in the greenhouse was readily obtained by spraying the plants with a water suspension of these bacteria in an atomizer. These plants were incubated under warm moist conditions and the spots appeared within three to six days. In the field the disease has likewise been produced by spraying the plants with the bacteria and here the spots became visible about five days after inoculation. Foliage infection of pepper and potato plants has also been secured in the greenhouse, while attempts to infect other relatives of the tomato such as tobacco, Jimson weed and ground cherry have been unsuccessful.

LEAF INFECTION THROUGH STOMATA

By a series of tests in which certain tomato plants were inoculated on the upper surfaces of the leaves and others on the lower surfaces, it was found that much more abundant infection was obtained when the lower epidermis was inoculated. Microscopic examination showed that stomata or breathing pores were much more abundant in the lower epidermis and since the bacteria gain entrance to the leaf through these stomata the

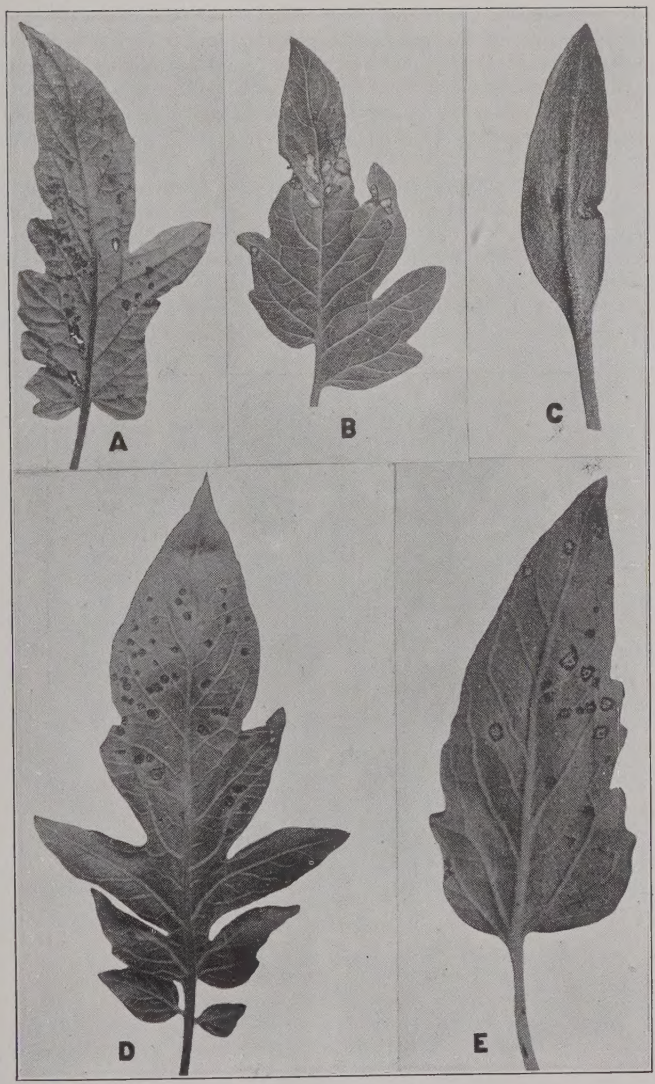

Fig. 6. Leaf infection.

A. Leaflet from a plant in the field showing cracked centers of the spots.
B. Distortion of young leaflet by the lesions.
C. Cotyledon lesions resulting from seed inoculation.
D. and E. Lesions resulting from inoculation in greenhouse.

greater susceptibility of the lower epidermis is due to the greater abundance of stomata therein. Cotyledons and very young leaves, however, may be readily infected through either surface and microscopic examination showed that stomata were abundant in both surfaces. Microscopic sections were prepared from an inoculated cotyledon and a clear-cut instance of a mass of bacteria within the leaf tissue just under one of these stomata was found.

After the bacteria gain entrance to the leaf tissue they multiply rapidly in the intercellular spaces or spaces between the cells of the leaf tissue and by filling up these spaces and driving out the air produce the water-soaked appearance of the lesion. Soon the leaf cells are killed and the diseased tissue darkens and dries out. The bacterial mass may ooze out to the surface of the leaf and produce a sticky exudate from which infection may be spread.

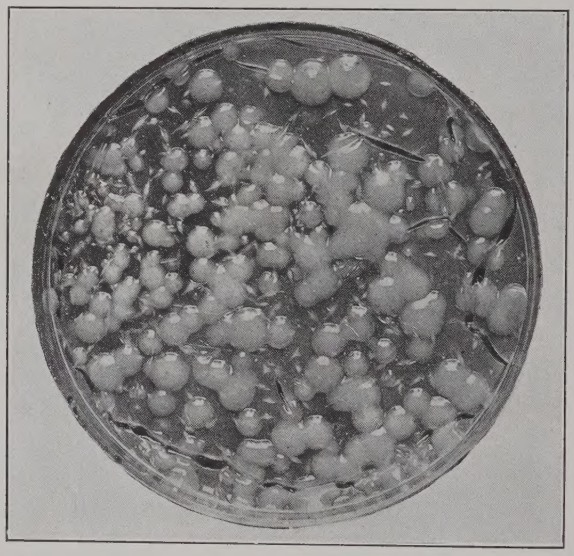

Fig. 7. Colonies of the bacterial spot organism in an agar plate.

FRUIT INFECTION THROUGH WOUNDS

When tomato fruits were sprayed with the bacteria no infection was obtained unless needle puncture wounds were made and no infection occurred in cases where unwounded fruits were wrapped in cotton which was kept continuously wet for several days with a suspension of the bacteria. Infection of green fruits was readily secured, however, by puncturing the epidermis and then spraying with a suspension of the bacteria, or by puncturing the fruit with a needle previously dipped in the bacteria. There are no stomata in the epidermis of the fruit so the presence of wounds is necessary for the bacteria to enter and produce fruit lesions.

Very minute wounds permit infection and successful inoculations were made with a very fine capillary glass point not over one millimeter in length.

Since fruit infection was obtained only through wounds it seemed quite likely that insects might be responsible for the wounds which permit infection in the field. Stink bugs, tarnished plant bugs and leaf-hoppers have been observed on tomato fruits. These are sucking insects which pierce the epidermis and suck the plant juices and the wounds which they make would be quite similar to the punctures through which artificial inoculation was successful. A number of these insects were confined in celluloid cages on the fruits (Fig. 9) of plants in the field after these fruits had been sprayed with the bacteria. Some infection was obtained with the stink bugs and tarnished plant bugs. This indicates that natural infection may occur through the feeding punctures of these insects. Natural infection may also occur through growth cracks, abrasions and the white spot or bird's-eye spot previously described.

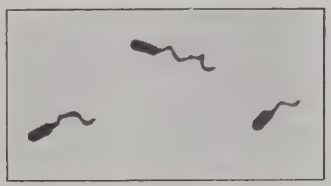

Fig. 8. Bacteria stained to show polar flagella. Photomicrograph; enlarged 2000 times.

In the greenhouse inoculation tests it was impossible to infect ripe fruits. This may be explained by the fact that the juice extracted from the tissue of ripe fruits was found to have a higher true acidity or hydrogen-ion concentration than that of the leaves and green fruits and higher than the bacteria will tolerate in culture media. On the other hand the true acidity of the green tissue was within the limits of tolerance of the bacteria.

In the fruit tissue the bacteria fill the intercellular spaces and cause a water-soaked appearance as in the leaf lesions. The upper layers of cells are killed while deeper-lying cells may be stimulated to enlarge and divide so that the center of the lesion is pushed upward to form an elevated scab (Fig. 10).

INOCULATED SEED YIELDS DISEASED SEEDLINGS

Tomato seed was dipped in a suspension of the bacteria, dried, and planted in soil flats in the greenhouse. On about 10 per cent of the seedlings, the large black lesions of bacterial spot appeared on the cotyledons (Fig. 6 C). Infection of the cotyledons probably occurred as these pulled out of the seed coats. These primary lesions differed quite distinctly from those produced by spraying the cotyledons in that they were larger, fewer in number, and were frequently associated with the adhering seed coat. Secondary infection of the cotyledons of other seedlings occurred as a result of the sprinkling process, but could be readily distinguished from the primary infection.

BACTERIA LIVE OVER WINTER ON THE SEED

In 1919 it was noted that the disease occurred rather uniformly in fields planted with seed from certain sources. This coupled with the fact that artificially inoculated seed gave rise to infected seedlings and the fact that the bacteria so successfully withstood drying on the surface of the seed led to a suspicion that the disease was carried over winter with the seed.

The infection on the fruit does not penetrate deeply but the process of seed extraction offers every opportunity for surface contamination of the seed. Each lesion contains millions of the bacteria and when the fruit is crushed these bacteria are dispersed into the juice and thus reach the seed.

Fig. 9. Tomatoes enclosed in a celluloid cage containing stink bugs.

The presence of hairs on the seed furnishes an immense surface area for the lodgment of bacteria and no amount of washing will remove all of these organisms. Laboratory tests have shown that these bacteria when dried on the seed coat survive a period of one year so infection might readily be thus carried from one season to the next.

Several lots of commercial seed saved from tomatoes grown in a large field where the disease was severe in 1919 were planted in sterilized soil in the greenhouse in April, 1920, and out of 6,887 plants 81 showed primary cotyledon lesions of bacterial spot. This proved that the causal organism was actually present on commercial seed and that the disease might thus be introduced into seed beds and new fields. One infected seedling in a hundred or even one in a thousand is enough to start an epidemic. Such a plant serves as a center of infection from which the disease spreads to others.

Tests made in February, 1921, with the same 1919 seed mentioned above proved that the organism also may be alive on two-year-old seed. Out of 1,321 plants, four showed primary infection. Thus the organism had survived 16 months on this seed.

Whether or not the organism may live over winter in the soil of plant beds or in the field has not yet been determined.

SEASONAL CYCLE OF THE DISEASE

The bacteria are present on the surface of the tomato seed, not of course in all lots of seed nor on all of the seed in any lot, and with contaminated seed the trouble is disseminated far and wide. When germination occurs the bacteria on the seed pass over through a film of water and infect the cotyledons as these pull out of the seed coat. From these primary cotyledon lesions infection is spread to other seedlings in the bed largely by the splashing of water. Foliage infection occurs through the

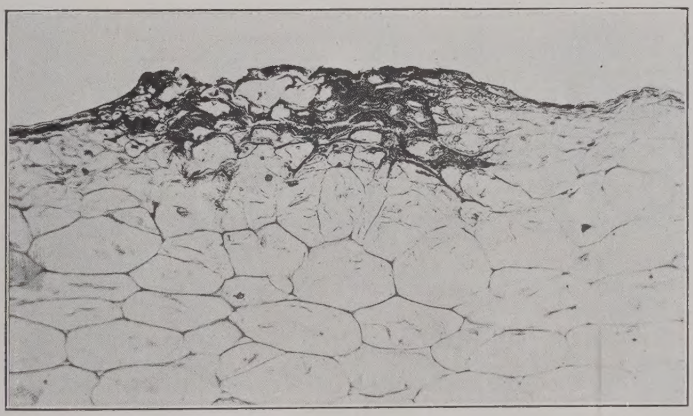

Fig. 10. Microscopic section through a fruit lesion.

stomata or breathing pores in the leaves. The disease is carried from the plant bed to the field with infected transplants and is widely disseminated by the sale and distribution of such transplants. Each diseased plant serves as a center of infection in the field from which the disease is spread to the foliage of other plants by the splashing and washing of rain. Later the bacteria from the foliage spots are splashed onto the fruit and fruit infection occurs through insect punctures and other wounds.

If there are tomatoes affected with bacterial spot among a lot from which seed is to be saved, some of this seed becomes contaminated with the bacteria which are dispersed from the fruit lesions into the juice when the fruit is crushed. The bacteria causing this disease are not killed when the seed is dried and some remain alive on the seed coat until planting time.

The life history of the causal organism may be thus traced: **Seed coat→cotyledon of seedling→foliage in plant bed→foliage in field→fruit→seed coat.**

CONTROL BY SEED DISINFECTION

Since the bacteria causing this disease are evidently carried on the seed, surface disinfection of the seed should kill these bacteria and thus prevent the appearance of original centers of infection in the plant bed and the field. In developing a suitable method of seed disinfection it was of course necessary to determine what treatments were harmless to the seed and at the same time effective against the bacteria.

To determine what methods might safely be used, small quantities of tomato seed were tied up in gauze bags and subjected to the treatments listed in Table I. Germination tests were then made in the local federal seed laboratory.

TABLE I.—Effect of Disinfection Upon Viability of Seed

Treatment	Percentage germination
Check, untreated	85
Hot water, 55° C. (131° F.) 10 minutes	68
Formaldehyde, 5 per cent, 5 minutes	89.5
Formaldehyde, 10 per cent, 5 minutes	89.5
Formaldehyde, 2.5 per cent, 50° C. (122° F.) 5 minutes	74.5
Corrosive sublimate 1 to 1000, 5 minutes	62.5
Corrosive sublimate 1 to 2000, 5 minutes	84
Corrosive sublimate 1 to 3000, 5 minutes	88
Corrosive sublimate 1 to 4000, 5 minutes	90

From these results it is evident that hot water, hot formaldehyde and corrosive sublimate, 1 to 1000, cannot be safely used, while the other treatments are harmless.

To test the effectiveness of these harmless treatments upon the bacteria, wet tomato seed was sterilized in the autoclave, inoculated with a suspension of the bacteria and dried. Small quantities of this seed were tied up in gauze bags, treated in the disinfectant, washed in sterile water and dried. Fifty to 100 seeds from each lot were then planted in agar plates. In case the bacteria on a seed were not all killed the characteristic colony of this organism appeared on the agar about this seed. The results are presented in Table II.

TABLE II.—Effectiveness of Seed Disinfection

Treatment	Percentage of seed showing bacterial growth	Per cent effectiveness
Check, untreated	98	
Formaldehyde, 5 per cent, 5 minutes	12	88
Formaldehyde, 10 per cent, 5 minutes	5	95
Check, untreated	92	
Corrosive sublimate 1 to 2000, 5 minutes	0	100
Corrosive sublimate 1 to 3000, 5 minutes	0	100
Corrosive sublimate 1 to 4000, 5 minutes	0	100

These results show that the formaldehyde is not reliable while the weaker strengths of corrosive sublimate are thoroughly effective. Repeated tests in which the seed was planted in soil flats proved that these weaker dilutions of corrosive sublimate are absolutely non-injurious to the seed. The percentage of germination was not reduced nor was the vigor of the seedlings impaired.

RECOMMENDATIONS

To control bacterial spot it is therefore recommended that tomato seed be treated in a 1 to 3000 solution of corrosive sublimate for five minutes and washed 10 to 15 minutes in running water. This treatment applies only to dry seed.

If small amounts of seed such as a pound or less are to be treated, the corrosive sublimate solution may be made by use of the proprietary tablets for sale in drug stores. If the directions are given for making a 1 to 1000 solution it will be necessary, of course, to use three times the amount of water specified.

When larger quantities of seed are to be treated, it is advisable to make up a concentrated stock solution by adding one ounce of corrosive sublimate to 22 pints of water or one pound to 44 gallons of water. The solution to be used on the seed is made up by adding one pint of the stock solution to one gallon of water, or one quart to two gallons and so on, depending upon the quantity needed. In making up this solution it is advisable to figure on at least one gallon to two pounds of seed.

The solution can be used only once and must be made up fresh for each bag of seed.

Metal containers can not be used. Glass, porcelain or wooden containers are satisfactory. For treating seed in lots of about six pounds each, five-gallon wooden candy buckets have proved convenient. For larger amounts of seed casks or barrels should be used.

The seed should be treated in a bag large enough to allow for the considerable swelling which takes place when dry tomato seed is moistened. During the treatment the bag of seed should be stirred thoroughly by poking with a stick so as to remove air bubbles and insure thorough wetting of the seed coats.

The bag of seed should be removed from the corrosive sublimate solution promptly at the end of five minutes and placed in another container in which water is allowed to run 10 to 15 minutes. After this thorough washing in running water, the surplus water should be squeezed out of the bag or allowed to drain out. The seed should then be spread out in a thin layer in a well ventilated place to dry. A clean floor space may be used for this purpose and small lots of seed may be spread on sheets of paper.

Precautions should be taken against recontamination of the treated seed and if old sacks or bags are to be used, these also should be dipped in the disinfectant.